

MICROBIAL GROWTH CONTROL

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Introduction



- Methods for inhibiting rapid microbial growth include **decontamination** and **disinfection**.
- Decontaminationis the treatment of an object or surface to make it safe to handle.
- Disinfection, in contrast, directly targets pathogens, although it may not eliminate all microorganisms.
- Specialized chemical or physical agents called disinfectants can kill microorganisms or inhibit microbial growth
- Physical methods are used in industry, medicine, and in the home to achieve microbial decontamination, disinfection, and sterilization.
- Heat, radiation, and filtration are commonly used to destroy or remove microorganisms.

Heat Sterilization



❑ **Measuring Heat Sterilization**

- Microorganisms lose viability at very high temperatures because most macromolecules lose structure and function, a process called *denaturation*
- The effectiveness of heat as a sterilant is measured by the time required for a 10-fold reduction in the viability of a microbial population at a given temperature.
- This is the decimal reduction time or D.

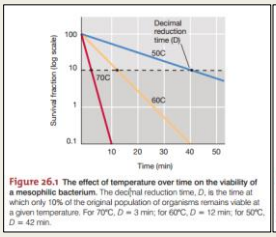


Figure 26.1 The effect of temperature over time on the viability of a mesophilic bacterium. The decimal reduction time, *D*, is the time at which only 10% of the original population of organisms remains viable at a given temperature. For 70°C, *D* = 3 min; for 60°C, *D* = 12 min; for 50°C, *D* = 42 min.

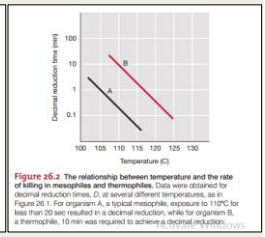


Figure 26.2 The relationship between temperature and the rate of killing in mesophiles and thermophiles. Data were obtained for decimal reduction times, *D*, at several different temperatures, as in Figure 26.1. For organism A, a typical mesophile, exposure to 112°C for less than 20 sec resulted in a decimal reduction, while for organism B, a thermophile, 10 min was required to achieve a decimal reduction.

Heat Sterilization

- The time and temperature, therefore, must be adjusted to achieve sterilization for each specific set of conditions.
- The type of heat is also important: **Moist heat** has better penetrating power than **dry heat** and, at a given temperature, produces a faster reduction in the number of living organisms
- An easier way to characterize the heat sensitivity of an organism is to measure the thermal death time, the time it takes to kill all cells at a given temperature.

Heat Sterilization

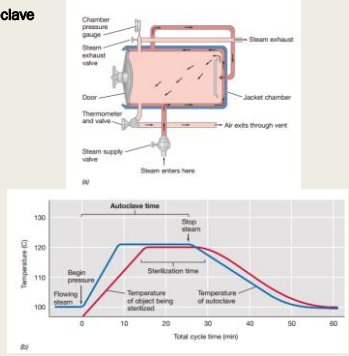
❑ **The Autoclave**


(c)

- The autoclaveis a sealed heating device that uses steam under pressure to kill microorganisms
- The autoclave uses a temperature of 121°C.
- At 121°C, the time to achieve sterilization of endospore-containing material is generally 10–15 minutes
- Extended times are also required when large volumes of liquids are being autoclaved because large volumes take longer to reach sterilization temperatures.
- Note that it is not the pressure inside the autoclave that kills the microorganisms but the high temperature that can be achieved when steam is applied under pressure.

Heat Sterilization

❑ **The Autoclave**



Heat Sterilization

❑ **Pasteurization**

- Pasteurization uses precisely controlled heat to reduce the number of microorganisms found in milk and other heat-sensitive liquids.
- At temperatures and times used for pasteurization of food products such as milk
- Pasteurization also controls commonly encountered pathogens such as *Listeria monocytogenes*, *Campylobacter* species, *Salmonella*, and *Escherichia coli*
- These pathogenic bacteria can be found in foods such as dairy products and juices
- Pasteurization of milk = 71°C for 15 seconds. This process is aptly called flash pasteurization.

Radiation Sterilization

❑ **Ultraviolet Radiation**

- Ultraviolet radiation between 220 and 300 nm in wavelength has enough energy to cause modifications or actual breaks in DNA, sometimes leading to disruption of DNA and death of the exposed organism
- This "near-visible" UV light is useful for disinfecting surfaces, air, and materials such as water that do not absorb the UV waves.
- For example, laboratory laminar flow hoods, designed to maintain clean work areas, are equipped with a "germicidal" UV light to decontaminate the work surface after use

Radiation Sterilization

❑ **Ionizing Radiation**

- Ionizing radiation is electromagnetic radiation of sufficient energy to produce ions and other reactive molecular species from molecules with which the radiation particles collide
- Ionizing radiation generates higher-energy electrons, hydroxyl radicals (OH) and hydride radicals (H) and each of these can damage macromolecules and kill irradiated cells

Radiation Sterilization

Table 26.1 *Radiation sensitivity of microorganisms and biological functions*

Species or function	Type of microorganism	$D10^a$ (Gy)
Clostridium botulinum	Gram-positive, anaerobic, sporulating *Bacteria*	3300
Clostridium tetani	Gram-positive, anaerobic, sporulating *Bacteria*	2400
Bacillus subtilis	Gram-positive, aerobic, sporulating *Bacteria*	600
Escherichia coli O157:H7	Gram-negative *Bacteria*	300
Salmonella typhimurium	Gram-negative *Bacteria*	200
Lactobacillus brevis	Gram-positive *Bacteria*	1200
Deinococcus radiodurans	Gram-negative, radiation-resistant *Bacteria*	2200
Aspergillus niger	Mold	500
Saccharomyces cerevisiae	Yeast	500
Foot-and-mouth	Virus	13,000
Coxsackie	Virus	4500
Enzyme inactivation		20,000–50,000
Insect deinfestation		1000–5000

^{a}D10 is the amount of radiation necessary to reduce the initial population or activity level 10-fold (1 logarithm). Gy = grays. 1 gray = 100 rads. The lethal dose for humans is 10 Gy.

- Table shows the dose of radiation necessary for a 10-fold (D10) in number of selected microorgan-isms.
- The D10 value is analogous to the decimal reduction time for heat sterilization

Radiation Sterilization

❑ **Radiation Practices**

- Common sources of ionizing radiation include cathode ray tubes that generate electron beams, X-ray machines, and radioactive nuclides 60_{Co} and 137_{Cs}, which are relatively inexpensive by-products of nuclear fission.
- These sources produce electrons (e^-), X-rays, or γ-rays, respectively, all of which have sufficient energy to efficiently kill microorganisms.
- In addition, X-rays and γ-rays pene-trate solids and liquids, making them ideal for treatment of bulk items such as ground beef or cereal grains.

Table 26.2 *Medical and laboratory products sterilized by radiation*

Tissue grafts	Drugs	Medical and laboratory supplies
Cartilage	Chloramphenicol	Disposable labware
Tendon	Ampicillin	Culture media
Skin	Tetracycline	Syringes
Heart valve	Atropine	Surgical equipment
	Vaccines	Sutures
	Ointments	

Table 26.3 *Recommended radiation dose for decontamination of selected foods*

Food type	kiloGrays
Fruit	1
Poultry	3
Spices, seasonings	30

Filter Sterilization

- Filtration accomplishes decontamination and even sterilization without exposure to denaturing heat.
- The liquid or gas is passed through a filter, a device with pores too small for the passage of microorganisms, but large enough to allow the passage of the liquid or gas.
- The selection of filters for sterilization must account for the size range of the contaminants to be excluded.

❑ **Depth Filters**

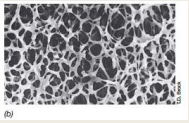
- A depth filter is a fibrous sheet or mat made from a random array of overlapping paper or borosilicate (glass) fibers
- The depth filter traps particles in the network of fibers in the structure
- Depth filters are also used for the filter sterilization of air in industrial processes.

Scanning electron micrograph showing the structure of a depth filter

Filter Sterilization

❑ **Membrane Filters**

- Membrane filters are the most common type of filters used for liquid sterilization in the microbiology laboratory
- Membrane filters are composed of high tensile strength polymers such as cellulose acetate, cellulose nitrate, or polysulfone, manufactured to contain a large number of tiny holes, or pores.



Scanning electron micrograph showing the structure of a membrane filter

Disposable, presterilized, and assembled membrane filter units.
Left: a filter system designed for small volumes.
Right: a filter system designed for larger volumes.

Chemical Growth Control

- An antimicrobial agentis a natural or synthetic chemical that kills or inhibits the growth of microorganisms.
- Agents that kill organisms are called **–cidal** agents, with a prefix indicating the type of microorganism killed. Thus, they are called **bacteriocidal, fungicidal**, and **viricida**
- Agents that do not kill but only inhibit growth are called **–static** agents. These include **bacteriostatic**, **fungistatic**, and **viristatic** compounds

❑ **Effect of Antimicrobial Agents on Growth**

- Viable cells are measured by plate counts
- The number of viable cells for a given organism is proportional to culture turbidity during the log phase of growth

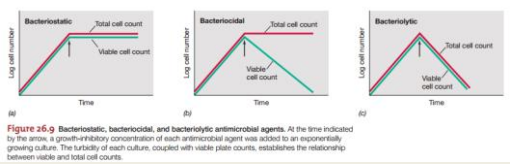


Figure 26.9 Bacteriostatic, bacteriocidal, and bacteriolytic antimicrobial agents. At the time indicated by the arrow, a growth-inhibitory concentration of each antimicrobial agent was added to an exponentially growing culture. The turbidity of each culture, coupled with viable plate counts, establishes the relationship between viable and total cell counts.

Chemical Growth Control

❑ **Measuring Antimicrobial Activity**

- Antimicrobial activity is measured by determining the smallest amount of agent needed to inhibit the growth of a test organism, a value called the **Minimum Inhibitory Concentration (MIC)**
- The MIC is not a constant for a given agent; it varies with the test organism, the inoculum size, the composition of the culture medium, the incubation time, and the conditions of incubation, such as temperature, pH, and aeration

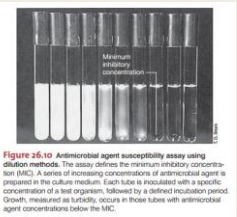


Figure 26.10 Antimicrobial agent susceptibility assay using dilution methods. The assay defines the minimum inhibitory concentration (MIC). A series of increasing concentrations of antimicrobial agent is prepared in the culture medium. Each tube is inoculated with a specific concentration of a test organism, followed by a defined incubation period. Growth, measured as turbidity, occurs in those tubes with antimicrobial agent concentrations below the MIC.

Chemical Growth Control

❑ Measuring Antimicrobial Activity

- Another common assay for antimicrobial activity is the disc diffusion technique
- A Petri plate containing an agar medium is inoculated with a culture of the test organism

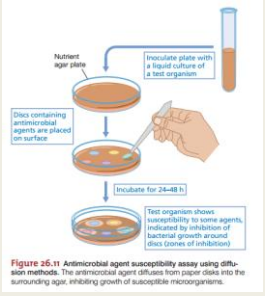


Figure 26.11 Antimicrobial agent susceptibility assay using diffusion methods. The antimicrobial agent diffuses from paper disks into the surrounding agar, inhibiting growth of susceptible microorganisms.

Antiseptic, Disinfectans, Antibiotics

❑ Antiseptic

- Antiseptics and germicidesare chemical agents that kill or inhibit growth of microorganisms and that are nontoxic enough to be applied to living tissues.

Agent	*Use*	*Mode of action*
Antiseptics		
Alcohol (60–85% ethanol or isopropanol in water)[a]	Topical antiseptic	Lipid solvent and protein denaturant
Phenol-containing compounds (hexachlorophene, triclosan, chloroxylenol, chlorhexidine)[b]	Soaps, lotions, cosmetics, body deodorants, topical disinfectants	Disrupts cytoplasmic membrane
Cationic detergents, especially quaternary ammonium compounds (benzalkonium chloride)	Soaps, lotion, topical disinfectants	Interact with phospholipids of cytoplasmic membrane
Hydrogen peroxide[a] (3% solution)	Topical antiseptic	Oxidizing agent
Iodine-containing iodophor compounds in solution[a] (Betadine®)	Topical antiseptic	Iodinates tyrosine residues of proteins; oxidizing agent
Octenidine	Topical antiseptic	Disrupts cytoplasmic membrane

Antiseptic, Disinfectans, Antibiotics

❑ Disinfectans

- Disinfectants are chemicals that kill microorganisms, but not necessarily endospores, and are used on inanimate objects.
- For example, disinfectants such as ethanol and cationic detergents are used to disinfect floors, tables, bench tops, walls, and so on.

Antiseptic, Disinfectans, Antibiotics

❑ **Disinfectans**

Agent	Use	Mode of action
Sterilants, disinfectants, and sanitizers[a]		
Alcohol (60–85% ethanol or isopropanol in water)[b]	Disinfectant for medical instruments and laboratory surfaces	Lipid solvent and protein denaturant
Cationic detergents (quaternary ammonium compounds, Lysol® and many related disinfectants)	Disinfectant and sanitizer for medical instruments, food and dairy equipment	Interact with phospholipids
Chlorine gas	Disinfectant for purification of water supplies	Oxidizing agent
Chlorine compounds (chloramines, sodium hypochlorite, sodium chlorite, chlorine dioxide)	Disinfectant and sanitizer for dairy and food industry equipment, and water supplies	Oxidizing agent
Copper sulfate	Algicide disinfectant in swimming pools and water supplies	Protein precipitant
Ethylene oxide (gas)	Sterilant for temperature-sensitive materials such as plastics and lensed instruments	Alkylating agent
Formaldehyde	3–8% solution used as surface disinfectant, 37% (formalin) or vapor used as sterilant	Alkylating agent
Glutaraldehyde	2% solution used as high-level disinfectant or sterilant, commonly used fixative in electron microscopy	Alkylating agent
Hydrogen peroxide[a]	Vapor used as sterilant	Oxidizing agent
Iodine-containing iodophor compounds in solution[a] (Wescodyne®)	Disinfectant for medical instruments and laboratory surfaces	Iodinates tyrosine residues
Mercuric dichloride[b]	Disinfectant for laboratory surfaces	Combines with –SH groups
OPA (ortho-phthalaldehyde)	High-level disinfectant for medical instruments	Alkylating agent
Ozone	Disinfectant for drinking water	Strong oxidizing agent
Peroxyacetic acid	Solution used as high-level disinfectant or sterilant	Strong oxidizing agent
Phenolic compounds[b]	Disinfectant for laboratory surfaces	Protein denaturant
Pine oils (Pine-Sol®) (contains phenolics and other detergents)	General disinfectant for household surfaces	Protein denaturant

Antiseptic, Disinfectans, Antibiotics

❑ **Antibiotics**

- Antibiotics are antimicrobial agents produced by microorganisms.
- Antibiotics are produced by a variety of bacteria and fungi and apparently have the sole function of inhibiting or killing other microorganisms.
- Gram-positive Bacteria and Gram-negative Bacteria differ in their susceptibility to an individual antibiotic such as penicillin;
- Gram-positive Bacteria are generally affected, whereas most Gram-negative Bacteria are naturally resistant.
- Certain **broad-spectrum antibiotics** such as **tetracycline**, however, are effective against both groups.

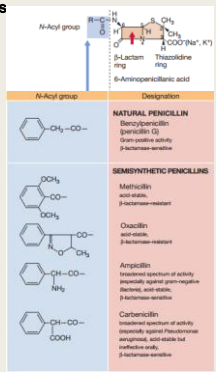
Antiseptic, Disinfectans, Antibiotics

❑ **Antibiotics**

- Antibiotics are antimicrobial agents produced by microorganisms.
- Antibiotics are produced by a variety of bacteria and fungi and apparently have the sole function of inhibiting or killing other microorganisms.
- One of the most important groups of antibiotics, both historically and medically, is the β-lactam group.
- β-lactam antibiotics include the medically important **penicillins** and **cephalosporins**
- **Penicillins**
- In 1929, the British scientist Alexander Fleming characterized the first antibiotic, an antibacterial compound called penicillin because it was isolated from the fungus *Penicillium chrysogenum*
- **Cephalosporins**
- Cephalosporins, produced by the fungus *Cephalosporium* sp., differ structurally from the penicillins.
- The cephalosporins have the same mode of action as the penicillins

Antiseptic, Disinfectans, Antibiotics

- ❑ Antibiotics
  - Penicillins



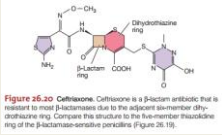


Figure 26.20 Ceftriaxone. Ceftriaxone is a β-lactam antibiotic that is resistant to most β-lactamases due to the adjacent six-member dihydrothiazine ring. Compare this structure to the five-member thiazolidine ring of the β-lactamase-sensitive penicillins (Figure 26.19).

Thank you ! ☺